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THE REGENERATION OF THE INTESTINAL EPITHELIUM IN THE TOAD (*BUFO LENTIGINOSUS AMERICANUS*) DURING TRANSFORMATION.*

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The phenomena of metamorphosis are perhaps best exemplified in the transformation of the tadpole into the frog or toad, or the caterpillar into the butterfly, and certainly are in these forms best known to the non-professional observer. To the biologist they present a field of investigation both suggestive and puzzling in its results. The remarkable changes of form and structure which occur necessitate a more or less complete distruction of parts with a subsequent regeneration to form the tissues of the adult body,—changes often comprised within a remarkably short period of time when it is considered how extensive they are.

In the higher orders of insects these processes of histolysis and regeneration are most extensive. In the frog and toad the changes easily recognized are the growth of arms and legs and the disappearance of the tail, whereby the animal is suited for a terrestrial instead of an aquatic existence. The alimentary tract likewise undergoes marked changes in preparation for the substitution of an animal for a vegetable diet. The mouth is widened, the horny beak is lost and true teeth developed; there occurs a differentiation of a stomach and rectum, accompanied by a marked shortening of the intestine as a whole. It is with the histolytic and regenerative changes in the intestinal epithelium that the present paper deals. In the yet untransformed tadpole, in which the legs are quite well developed but before the arms have yet broken through, the intestinal epithelium presents the appearance characteristic of the Amphibia: it possesses a simple columnar epithelium, thrown into folds when the intestine is contracted. Connected with the epithelium and hanging down from it or partially intercalated between the bases of the columnar cells are clusters of three or four small cells with scanty protoplasm, which have been generally homologized with the crypts of Lieberkühn of mammals. A lumen is but rarely to be recognized in these clusters in Amphibia, though an arrangement of the cells as if surrounding a lumen may be quite often found. In the tadpole, however, such is not the case, and in younger tadpoles cell-clusters as such apparently do not exist. Their origin and first appearance have not been ascertained as yet.

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*The following note is a fragment of a more extensive investigation upon the minute structural changes that occur in the toad during the period of transformation into the adult, and was undertaken in connection with work by Professor Gage of Cornell upon the habits and life-history of the animal. The colored figures by which this article was illustrated when read will be published with the final paper.

At a later stage, after the arms have been put forth and before there is any appreciable diminution in the size of the tail, these cell-clusters have increased markedly in size, and karyokinetic figures are abundant in them. From these cell-clusters the new epithelium of the transformed toad will be formed and the changes that lead to the final establishment of the adult intestine now follow rapidly, so that, before the tail is more than half absorbed, the new epithelium is completely formed and the old, larval epithelium occupies the lumen of the intestine as a disorganized mass of matter, cell fragrants, nuclei and globules; many of them apparently containing substance of a fatty nature which is stained black by means of osmic acid solutions.

The degeneration of the old epithelium is first indicated by the browner stain given to the protoplasm by osmic acid mixtures such as Hermann's and Flemming's fluids; globules and granules of a brown and black color-reaction with osmic acid appear in the cell body, which becomes vacuolar. The epithelium then begins to disintegrate and cell-outlines become indistinguishable. The nuclei remain for a long time apparently unchanged and may be easily distinguished after the cell bodies are completely destroyed. Accompanying these degenerative changes in the surface epithelium has been the steady growth of the cell-clusters or crypts. At first they are simply solid balls of cells; soon, however, a lumen appears in the middle of the ball and it becomes converted thereby into a hollow sphere or often a flask whose wall is a single layer of columnar cells. The spheres grow, the side toward the old epithelium opens, the neighboring crypts (spheres) meet one another and fuse, and in that manner a new continuous epithelium is formed. The old disintegrating epithelium is apparently simply displaced, —*pushed* into the lumen of the intestine where it remains as a mass of debris.

The bearing these facts have on the difficult question of the mode of regeneration of the intestinal epithelium is quite interesting and suggestive. At the present day histologists are confronted by two theories as to the mode of regeneration of the intestinal epithelium,—the first, which may be styled the theory of *Erbstein*, which is the older view, is that the new epithelial cells may be formed from the small cells which have been found here and there lying between the bases of the columnar cells, and which are generally spoken of as substitution cells,—a prejudgement of their nature and function. On the other hand, Bizzozero affirms that the crypts of Lieberkühn are the seat of regenerative activity and from their depths proceed new cells to accommodate an expanse of surface or replace cells which have been lost by abrasion. There are several investigators whose results show at least that karyokinesis is much more abundant in the crypts of Lieberkühn of mammals than in the surface epithelium or on the villi,—circumstantial evidence in favor of Bizzozero's theory. The migration of cells from the crypts to the summit of the villi which this theory presupposes, is, it seems to me, rather hard to accept without strong evidence.

Granting the correctness of the homology of what I have spoken of as cell-clusters in Amphibia with the crypts of Lieberkühn in mammals, Bizzozero's theory would find in the regeneration of the epithelium in the toad during transformation an argument in favor of its correctness, since here the new epithelium is entirely formed from the so-called glands or crypts of Lieberkühn.

It is likewise interesting to note that we encounter here in Amphibia quite the same mode of regeneration of the epithelium that has previously been found by other investigators to occur in insects during metamorphosis, and in dragon-fly nymphs at least, to supply loss of cells during normal digestive activity, as the results of some recent investigations (Needham) have shown.

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